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(54) Title: USE OF THIOREDOXIN, THIOREDOXIN-DERIVED, OR THIOREDOXIN-LIKE DITHIOL PEPTIDES IN HAIR CARE PREPARATION

(57) Abstract

The subject invention enables a more efficient management of hair by providing a novel preparation for waving, straightening, softening, or removing hair, employing as a key ingredient the compound thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide in combination with an organic reducing agent, or a sulfite or bisulfite compound. This invention allows hair to be treated at a lower pH to minimize hair damage when waving, straightening, or softening the hair; when used to remove hair, objectionable odors of commercial depilatories are minimized or eliminated.

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DESCRIPTION

USE OF THIOREDOXIN, THIOREDOXIN-DERIVED, OR THIOREDOXIN-LIKE DITHIOL PEPTIDES IN HAIR CARE PREPARATIONS

Background of the Invention

The care of hair has been of utmost importance to mankind from the beginning of recorded history. The reign of Queen Elizabeth (1558-1603) became noted for its attention to the finer aspects of hair styling; it was Her Majesty who set the standard. During this Elizabethan period, hair was arranged in elaborate high coiffures, and curled and frizzed by whatever means were available. Needless to say, as measured by present day available hair care products and methods, the Elizabethan hair care procedures were primitive, at best. The discovery of new chemicals and properties thereof led to the beginning of hair care products designed to beautify and maintain the hair in a healthy, youthful state. These desirable human hair properties were achieved by use of a variety of hair care products, including hair dyes and products used to impart a wave to the hair. Wavy hair is considered a desirable human hair feature, whereas straight hair is usually held in less favor. Because of these human demands to beautify the hair, there has evolved a multitude of hair care products with a variety of claims and promises. With hair care products designed to dye or wave the hair, it has been found that the structure of the hair shaft itself must be reckoned with in order to have a product which would give the desired results. A key detail of the hair shaft, which is predominantly keratinaceous in nature, is that the keratin fibers are bonded together by disulfide crosslinkages. It is this detail of the hair structure which the subject invention is concerned with. The prior art discloses the severance of the disulfide crosslink with, enter alia, various chemical agents.

Perhaps the most widely used chemicals, which are referred to as reducing agents since the disulfide crosslink is converted to sulfhydryl groups, are organic reducing agents such as thioglycolic acid or thiolactic acid. Recognized limitations of these current organic reducing agent-based waving formulations include their sensitivity to air oxidation, their inherent unpleasant odor, and their marginal efficacy at neutral pH. The general requirement for highly alkaline conditions (pH 8.5) combined with high thiol concentration is responsible for hair damage described as "overprocessing."

In a normal "tepid" waving process, keratin disulfides are reduced by use of either sulfites or bisulfites. Sulfite and bisulfite waving have the advantage of being less damaging than a thioglycolate wave with less likelihood of overprocessing. Drawbacks to the sulfite and bisulfite waving process however, are that they give soft waves and the permanent does not last as long as the thioglycolate wave. An additional detraction of the bisulfite wave is the formation of keratin thiosulfates, commonly known as Bunte salts. The presence of residual Bunte salts can lead to relaxation of the curl through disulfide exchange and to lanthionine formation causing irreversible hair damage. These Bunte salts can also affect the texture and feel of hair.

Brief Summary of the Invention

The subject invention concerns the surprising and advantageous discovery that the use of organic reducing agents such as thioglycolic acid, L-cysteine ethylester, beta-mercaptoethylamine, cysteine, mercaptosuccinic acid, beta-mercapto propionic acid, dimercapto adipic acid, thiomalic acid, thioglycollamides, glycol thioglycollate, glycerol thioglycollate, thiolactic acid and salts thereof (referred to collectively as organic reducing agents) in hair care preparations can be dramatically improved upon by use of thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide in combination with organic reducing agents. This

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combination gives rise to a synergistic effect in terms of efficiently breaking the disulfide bond of hair keratin. The net result is that significantly lesser amounts of organic reducing agents are needed to produce the desired effect in the hair. Coupled with this reduction of organic reducing agent use are other desirable features: The hair can be treated at a lower pH to minimize hair damage, and objectionable odors are minimized or eliminated. In achieving these desirable results, the subject invention enhances rather than compromises the reductive properties of organic reducing agents and additive dithiol peptides.

A further aspect of the subject invention is the discovery that the use of sulfite or bisulfite compounds in hair care preparations can also be improved upon by use of thioredoxin or thioredoxin-derived, or thioredoxin-like, dithiol peptide compounds.

The use of thioredoxin compounds significantly reduces the amount of sulfite or bisulfite compounds needed to achieve the desired effect in the hair. This combination of thioredoxin compounds with sulfites or bisulfites facilitates waving the hair in a shorter time and makes it possible to cleave the Bunte salts formed from the hair fiber. Thus, the use of thioredoxin compounds enhances the reductive properties of sulfite and bisulfite compounds and additive dithiol peptides.

Detailed Disclosure of the Invention

Upon adding thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide to a hair care product containing an organic reducing agent, e.g., a preparation for straightening, waving, removing, or softening hair, there is a realized synergistic effect whereby significantly lesser amounts of organic reducing agents are needed to produce the desired effect. For example, in commercial practice thioglycolic acid (TGA) is used in waving lotions at about 0.6 M (Molar) concentration. As the TGA concentration increases from about 0.01 M to about

0.6 M, the amount of hair curling increases, with a leveling off occurring at about 0.4 M TGA. By adding thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide to the waving preparation, the TGA concentration can be reduced by a factor of 4 (to about 0.15 M) and still give the same amount of waving as a commercial waving lotion containing 0.6 M TGA. This effect carries over to the other organic reducing agents also.

A synergistic effect is also observed when thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide is added to a hair care product containing a sulfite or bisulfite compound. By adding the thioredoxin compound, significantly less bisulfite or sulfite is needed to effect the desired hair treatment.

For example, in commercial practice, sodium or ammonium bisulfite is used in waving lotions at about 7.0% concentration levels. As the bisulfite concentration increases from about 0.01% to about 7.0%, the amount of hair curling increases with a leveling off occurring at about 7.0%. By adding thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide to the waving preparation, the bisulfite concentration can be reduced by a factor of two to about 3.2% and still give the same amount of waving as a commercial waving lotion containing 7.0% bisulfite.

The concentration of thioredoxin or one of the thioredoxin-derived, or thioredoxin-like, dithiol peptides which can be used to enhance the effect of an organic reducing agent, or a sulfite or bisulfite compound, ranges from about 1 to about 100 nmole/ml. The optimal concentration for intact bacterial thioredoxin appears to be about 2 nmole/ml. It should be recognized that the precise level of thioredoxin or thioredoxin-derived, or thioredoxin-like, dithiol peptide in combination with an organic reductant, or a sulfite or bisulfite compound, can be readily ascertained for a particular hair sample by a person skilled in the hair care art having possession of the subject invention.

Thioredoxins are low molecular weight dithiol proteins that have the ability to reduce disulfides in typical organic compounds such as Ellman's reagent or disulfides as they exist naturally in a variety of proteins (Holmgren, A. [1981] Trends in Biochemical Science 6:26-39).

Thioredoxin and thioredoxin-derived, or thioredoxin-like, dithiol peptides within the scope of the subject invention are exemplified by the following compounds:

(1) thioredoxin isolated from Escherichia coli (Laurent, T.C., Moore, E.C., and Reichard, P. [1964] J. Biol. Chem. 239:3436-3445);

(2) thioredoxins isolated from other sources, e.g., thioredoxin isolated from yeast (Porque, G.P., Baldesten, A., and Reichard, P. [1970] J. Biol. Chem. 245:2362-2379); Cyanobacterium (Gleason, F.K. and Holmgren, A. [1983] in "Thioredoxins, Structure and Function" [P. Gadal, ed.] Editions du Centre National de la Recherche Scientifique); rat (Guerara, J., Moore, E.C., and Ward, D. NM. [1983] ibid); T_4 bacteriophage (Soderberg, B.-O., Sjoberg, B.-M., Sonnerstam, U., and Branden, C.-I. [1978] Proc. Natl. Acad. Sci. USA 75:5827-5830); purification of mammalian thioredoxin (Luthman, M. and Holmgren, A. [1982] Biochem. 121:6628-6633); further, thioredoxin from a human source can be used in the subject invention;

(3) thioredoxin-derived dithiol peptides representing peptides produced by cleavage of intact thioredoxins, as described in Example 2, infra. One such example of this class of thioredoxin-derived peptides is the fragment containing residues 1 through 37 (i.e., T_{1-37}) produced by cyanogen bromide cleavage of thioredoxin from E. coli. The important feature of these thioredoxin-derived dithiol peptides is that they contain the redox-active peptide sequence, Cys-X-Y-Cys, wherein

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X and Y, independently, can be any of the natural 20 amino acids. For example, the redox-active peptide sequence from E. coli thioredoxin is Cys-Gly-Pro-Cys (Cys=cysteine, Gly=glycine, Pro=proline). Also the redox-active sequences Cys-X-Y-Cys-Lys or Trp-Cys-X-Y-Cys-Lys, wherein X and Y are as defined above, for example, Cys-Gly-Pro-Cys-Lys or Trp-Cys-Gly-Pro-Cys-Lys can be used; and

(4) thioredoxin-like dithiol peptides that inter alia have the intrinsic ability to catalyze the reduction of protein disulfides. These thioredoxin-like dithiol peptides will generally have the characteristic of containing a pair of cysteine residues which form a redox-active disulfide. This example includes peptides, derived from natural sources or constructed synthetically, that include the same redox-active sequence as disclosed above, for example in E. coli thioredoxin, Cys-Gly-Pro-Cys, Cys-Gly-Pro-Cys-Lys, or Trp-Cys-Gly-Pro-Cys-Lys, or analogous sequences from other thioredoxins such as that encoded for by T4 bacteriophage, Cys-Val-Tyr-Cys (Cys=cysteine, Val=valine, Tyr=tyrosine) (Soderberg, B.-O., Sjoberg, B.-M., Sonnerstam, U., and Branden, C.-I. [1978] Proc. Natl. Acad. Sci. USA 75:5827-5830). Other thioredoxin-like peptides include the class of seed proteins called purothionins that have intrinsic thioredoxin-like activity (Wada, K. and Buchanan, B.B. [1983] in "Thioredoxins, Structure and Function" [Gadal, P., ed.] Editions du Centre National de la Recherche Scientifique).

Following are examples which illustrate products of the invention and procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Permanent Waving of Hair

(a) Commercial Hair Waving Lotion and TGA

Many commercial hair waving lotions contain as the active ingredient thioglycolic acid. TONI SILKWAVE™ (for normal hair) (Trademark of Gillette, Boston, Massachusetts) is one such preparation and was used as a reference. A solution of 0.6 M sodium thioglycolate, 0.6 M ammonium bicarbonate, pH 8.6, was also used as a control for all experiments. Table 1 shows that the 0.6 M TGA solution gave results comparable to the commercial preparation.

Table 1. Permanent Waving with TONI SILKWAVE™ and 0.6 M Thioglycolic Acid

Waving Lotion	Relative Hair Length[1]
TONI™	0.80
0.6 M TGA	0.79

[1]Relative Hair Length was determined as follows:

Hair tresses were divided into small tresses approximately 1 cm wide.

Each tress was treated with 2 ml of waving lotion. Half of the waving lotion (1 ml) was applied to the tress and combed through. An end paper was folded around the tress to assure that the tress was flat and that all ends were covered. The tress was rolled firmly and evenly on the smallest rods available in the TONI SPIN CURLER™ assortment. After the tress was rolled the remaining waving lotion was applied and the tress kept for 15 minutes at room temperature. After 15 minutes the tress was rinsed for 30 seconds under warm running tap water, blotted dry, and maintained at room temperature for an additional 30 minutes.

The tress was neutralized by applying 2 ml of hydrogen peroxide (0.5%). After 3 minutes, the curling rod was removed and an additional 2 ml of hydrogen peroxide was applied. The tress was rinsed for 30 seconds under warm running tap water, blotted dry, and allowed to completely air-dry before making any measurements.

Differences in the amount of waving from the different solutions were quantitated by measuring the hanging length before and after waving. The relative hair length was calculated as shown in the following equation:

$$RHL = \frac{L_a}{L_b}$$

where RHL is the relative hair length, L_b the length before waving, and L_a the length after waving.

(b) Influence of TGA

TGA concentration ranging from about 0.01 M to about 0.6 M was evaluated in a solution containing ammonium bicarbonate (NH_4HCO_3) in the same molar concentration as TGA at pH 8.6. The influence on waving was determined in the absence and presence of 10 nmole/ml of thioredoxin. Two hair lots were also used. The tresses were waved and the relative hair length determined as described above. The results showed that as the TGA concentration increased from about 0.01 M to about 0.6 M the amount of curling also increased and leveled off at approximately 0.4 M.

In the presence of thioredoxin (10 nmole/ml) there was an increase in the amount of waving measured at each concentration of TGA used. At higher TGA concentrations this effect was more pronounced and could be easily visualized. At 0.2 M TGA the amount of waving in the presence of thioredoxin was greater than that of either 0.6 M TGA alone or the TONI™ waving lotion. With

thioredoxin present the TGA concentration could be reduced by a factor of 4 (to 0.15 M) and still give the same amount of waving as the commercial waving lotion.

(c) Influence of Thioredoxin

The influence of thioredoxin on permanent hair waving at various TGA concentrations was tested with thioredoxin concentrations ranging between about 10 and about 100 nmole/ml. Thioredoxin was added to the waving solution, mixed well, and applied to hair tresses as described above. The waving solutions used were: (1) 0.1 M TGA, 0.1 M NH_4HCO_3, pH 8.6, (2) 0.2 M TGA, 0.2 M NH_4HCO_3, pH 8.6, and (3) 0.4 M TGA, 0.4 M NH_4HCO_3, pH 8.6. The results showed that a thioredoxin concentration of 10 nmole/ml gave maximal waving.

In a second test, thioredoxin concentrations were varied from about 1 to about 10 nmole/ml. The waving solution was solution (2) above. The results showed that thioredoxin concentration can be decreased to as low as 2 nmole/ml before there is a noticeable decrease in the effect on waving.

Influence of Other Monothiols

Alternate monothiols were tested for waving with and without thioredoxin with the hopes of finding an alternative reducing agent for thioredoxin that would complement waving. Monothiols with pK_a's ranging from 7 to 11 were tested at both pH 7.0 and 8.6 for waving activity. As the pK_a decreases, the amount of waving increases at both pH 7.0 and 8.6. Thioredoxin influences the waving of all the monothiols tested at both pH 7.0 and 8.6 and the effect is similar to that observed with TGA.

Several monothiols were more effect at 0.2 M than the commercial preparation containing TGA. More waving was achieved with the various monothiols upon the addition of 2uM thioredoxin. The amount of L-cysteine ethylester needed in the presence of thioredoxin to give the same waving as the commercial solution was 0.1 M.

(d) Influence of pH

One disadvantage of the commercial permanent waving preparations is the high pH. Thus, an object of the subject invention is to be able to lower the pH and still obtain a high level of waving. When the pH was decreased from 8.5 to 7.5 there was also a decrease in the amount of waving, both in the presence and absence of thioredoxin. At pH 7.5, however, the amount of waving in the presence of thioredoxin and 0.2 M TGA was still greater than the amount of waving with the 0.2 M TGA alone at pH 8.6. At pH near neutral, TGA becomes a less efficient reducing agent, contributing to the decreased waving, both in the presence and absence of thioredoxin.

The pH was adjusted with concentrated HCl. The waving solutions contained 0.2 M TGA and 0.2 M NH_4HCO_3, and 0.2 M TGA, 0.2 M NH_4HCO_3, and 40 nmole/ml thioredoxin. The experimental procedure was as described above.

Example 2 – Production of Purified Thioredoxin

Thioredoxin is purified either from a commercial source of E. coli, strain B (Grain Processing Corp., Minneapolis, MN) or from any of a number of common strains of E. coli grown by standard procedures (Pigiet, V. and Conley, R.R. [1977] J. Biol Chem. 252:6367-6372). The protein is purified using standard procedures including chromatography on ion exchange and molecular sieve columns (Williams, C.H., Zanetti, G., Arscott, L.D., and McAllister, J.K. [1967] J. Biol. Chem. 242:5226-5231; and McEvoy, M., Lantz, C., Lunn, C.A., and Pigiet, V. [1981] J. Biol Chem. 256:6646-6650). Thioredoxin was at least 95% homogeneous as determined by SDS-polyacrylamide gel electrophoresis. The enzyme was stored in 5 ml aliquots in -20°C in 0.5M Tris, pH 7.4 with 1 mM EDTA.

Thioredoxin protein is assayed immunologically using quantitative rocket immunoelectrophoresis as described in McEvoy et al., supra.

Example 3 – Production of Thioredoxin Fragments

(a) Production of T_{1-37} by Cyanogen Bromide Cleavage

A sample of E. coli thioredoxin was dialyzed in water for 12 hr at 4°C. Five ml was dried and resuspended in 70% formic acid. Cyanogen bromide (Sigma Chemical, St. Louis, MO) was dissolved in 70% formic acid and added to thioredoxin in a 50-fold molar excess of methionine. The solution was purged with nitrogen and incubated at room temperature in the dark for 24 hr. At the completion of the cleavage reaction the solution was dried under nitrogen, resuspended in sodium acetate buffer and adjusted to pH 8.5 with ammonium hydroxide.

When the sample was dissolved, the pH of the sample was adjusted to 8.0 with concentrated HCl. The sample was stored at -20°C under argon and aliquots were removed for purification.

T_{1-37} was isolated by affinity chromatography on thiopropyl sepharose 6B. A sample of the CNBr digest was incubated with a 2-fold molar excess of DTT for 10 minutes at room temperature before being applied to a thiopropyl column equilibrated with 0.1M Tris, pH 7.5, containing 0.5M NaCl and 1 mM EDTA. The column was washed with two column volumes of the equilibrating buffer containing 2M urea to remove any T_{38-108} that was non-specifically bound. The column was then washed with an additional 2 column volumes of equilibrating buffer. T_{1-37} was eluted from the column with 25mM DTT in equilibrating buffer. The sample was analyzed for homogeneity by reverse phase high pressure liquid chromatography on a Waters u-Bondpak C_{18} column attached to a Beckman Model 421 system monitored at 214 nm. A 0-60% gradient of acetonitrile containing 0.08 to 0.1% TFA (Buffer B) was used to elute the peptide at a flow rate of 2 ml/min. The peptide was judged to be greater than 95% pure by this procedure.

DTT was removed from the sample by exclusion chromatography. The sample volume was reduced using a Savant Speed Vac Concentrator and applied to a 1 cm x 25 cm column of Sephadex™ G-25-40 equilibrated with 0.05M Tris, 1mM EDTA, pH 7.4 (TE buffer). The 0.3 ml fractions collected were monitored at 280 nm. The samples containing T_{1-37} were pooled and the concentration determined by A_{280} ($_{280}$ = 10,000 $cm^{-1}M^{-1}$). The sample was immediately used in the waving assay.

(b) Production of T_{19-36} by Trypsin Cleavage

After HPLC separation, described above, T_{1-37} was pooled, dried, and resuspended in sodium acetate buffer and adjusted to pH 8.0 with NH_4OH. An aliquot of trypsin (Sigma Chemical) was added to the incubation at 1% (w/w) of peptide concentration. The reaction mixture was incubated at 37°C for 1 hr. Separation of trypsin fragments was done by HPLC as for the cyanogen bromide fragments.

Trypsin digestion of the T_{1-37} peptide yielded two peptides, T_{4-18} and T_{19-36}, which were resolved by HPLC, eluting at 31% and 45% in 0.08% trifluoroacetic acid in acetonitrile (Buffer B), respectively. Amino acid analysis revealed that the species eluting at 31% B contained 15 amino acids and corresponds to the active site peptide, T_{19-36}. Incubation of 90 nmoles of T_{1-37} produced 80 nmoles of T_{19-36} after separation by HPLC with a yield of 88%.

Residues 19 through 36 can be used in the same manner as residues 1-37, described herein.

(c) Isolation of T_{32-37}: T_{32-37} was isolated from a chymotryptic digest of the CNBr digest. Chymotrypsin was added to a prewarmed (37°C) solution of CNBr digest to a final concentration of 1:20 (w/w) chymotrypsin to peptide. After incubating the sample for 1 hr L-1-Tosylamide-2-phenylethylchloromethyl ketone (TPCK) was added in a 1:1 molar ratio to chymotrypsin to stop the reaction.

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The sample was loaded onto a Waters u-Bondpak C_{18} column attached to a Beckman Model 421 system monitored at 214 nm. The solvent system employed was 0.1% trifluoroacetic acid (Buffer A) and 0.08% trifluoroacetic acid in acetonitrile (Buffer B). A gradient from 0-30% B over 30 minutes and 30-60% over 15 minutes was used to separate the peptides at a flow rate of 2 ml/min. The peak identified as T_{32-37} was collected, taken to dryness, and stored under argon at -20°C.

(d) Reduction of T_{31-36}: T_{31-36} was obtained synthetically and was reduced for several experiments. The peptide was incubated at 37°C for 1 hr with a 5-fold molar excess of DTT. The DTT was removed by HPLC as described for the isolation of T_{32-37}. T_{31-36} was taken to dryness, reconstituted with a minimal volume of TE buffer and used immediately in the waving assay. The procedure was only partially effective, with only 30% of the peptide reduced as determined by cleavage of a model Bunte salt.

Example 4 - Influence of Fragment T_{1-37} on Permanent Hair Waving

The effect of T_{1-37} peptide on waving is shown in Table 2. The peptide has a comparable influence on waving as the intact thioredoxin. In this study the concentration used was twice as great as that of the intact thioredoxin. In a second test T_{1-37} peptide was varied from about 1 to about 80 nmoles/ml. The waving solution is the same as described in Table 2. The results showed that the T_{1-37} peptide can be decreased to as low as 1 nmole/ml before there is any noticeable decrease in the effect on waving.

Table 2. Influence of Fragment T_{1-37} on Permanent Hair Waving

Waving Lotion	Relative Hair Length A	B
0.2 M TGA	0.84	0.81
0.2 M TGA + Thioredoxin[1]	0.79	0.73
0.2 M TGA + T_{1-37}[2]	0.79	0.73

[1]The thioredoxin concentration in both A and B was 10 nmole/ml.

[2]The T_{1-37} fragment concentration was 26 nmole/ml for A and 20 nmole/ml for B. A and B were run on two different days using two different lots of hair. The pH for all assays was 8.6.

Example 5 - Bisulfite Waving Solutions

The bisulfite waving solution consisted of 7% (w/w) ammonium bisulfite, 4.65% (w/w) ethanol, and 0.6% (w/w) polyoxyethylene(23) lauryl ether. The pH was adjusted to 7.5 with ammonium hydroxide. All dilutions of the 7% solution were made using a diluent consisting of all components except the ammonium bisulfite. The neutralizer contained 2.3% hydrogen peroxide adjusted to pH 3.3 with dilute phosphoric acid. All solutions were overlayed with argon or nitrogen and stored in the dark at room temperature for up to 2 months.

Hair Waving Assay: The influence of thioredoxin and active site peptides on bisulfite permanent waving was determined by direct hair waving assays. Tresses were divided into smaller tresses approximately 0.5 cm wide and 12 to 13 cm in length. Each tress weighed approximately 0.2 g.

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Intact tresses were shampooed before waving with SILKIENCE™ for normal hair (The Gillette Company, Boston, MA). Each tress was shampooed and thoroughly rinsed two times before being combed through and allowed to air dry. Bleached-waved tresses were used as received with no additional treatment.

Each tress was treated with a total of 3 ml of waving lotion. Half the lotion (1.5 ml) was applied to the tress and the saturated tress remained at room temperature for 20 minutes before being combed through and rolled. An end paper was folded around the tress to assure that the tress was flat and all ends were covered. The tress was rolled firmly and evenly on the medium orange rods (0.6-0.7 mm diameter) available in the RAVE™ curler assortment (Chesebrough-Pond's Inc., Trumbull, Connecticut). After the tress was rolled it remained at room temperature for an additional 20 minutes. The tress was then saturated with the remaining waving lotion, covered with plastic wrap, and incubated for 60 minutes at 33-34°C. After 60 minutes the tress was rinsed for 3 minutes with 40°C tap water, blotted dry, and neutralized.

The tress was saturated with neutralizer and resaturated after 90 seconds. After 10 minutes at room temperature, the rod was removed from the tress and the neutralizer worked down to the end of the tress. After an additional 2 minutes the tress was rinsed for 2 minutes with 40°C tap water and the hanging length was measured immediately while the tress was wet.

Differences in the amount of waving from the different solutions were quantitated as described in Example 1(a).

Example 6 – Influence of Thioredoxin

Thioredoxin was effective in increasing the waving obtained from a bisulfite solution in hair. As anticipated, the amount of waving with bisulfite alone was significantly less in intact hair (RHL = 0.74) as compared to bleached-waved hair (RHL = 0.70). The increase in waving with the addition of thioredoxin was also

more significant. The RHL with the addition of thioredoxin was 0.69 for intact hair compared to 0.68 for bleached-waved hair. Thioredoxin in 3.2% bisulfite produced more waving than a 7% commercial preparation (CLAIROL KINDNESS™). The amount of dithiol required to give the maximal effect was 2-5uM.

The addition of thioredoxin affected the processing time required in treated hair. The presence of thioredoxin significantly reduces the observed biphasic nature of the reaction. For example, in intact hair, thioredoxin increased the amount of waving at a reaction time as short as 15 minutes. Intact tresses were waved with a 3.2% bisulfite solution alone or containing 5 uM thioredoxin. As with treated hair, the greatest increase in waving with the addition of thioredoxin was at 45 minutes.

These results indicate two important conclusions. First, with the addition of thioredoxin the amount of waving in a bisulfite system can be increased. Second, if no increase in the amount of waving is desired, the processing time can be reduced from 60 minutes to 40-45 minutes with the addition of thioredoxin to a 3.2% bisulfite solution.

Example 7 – Influence of Thioredoxin and Cysteine Methyl Ester

Studies on the cleavage of a model Bunte salt with thioredoxin supported the idea that at low pH a stable sulfonated thioredoxin intermediate is formed and this intermediate could be recycled to reduced thioredoxin by the addition of a secondary reductant such as cysteine methyl ester or cysteine. Above neutral pH this intermediate is unstable and oxidized thioredoxin is formed.

The effect of reduced thioredoxin (5 uM) and various concentrations of cysteine methyl ester on permanent waving at neutral pH was determined. Cysteine methyl ester in the absence of thioredoxin increased the amount of

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waving though it was not as effective as intact thioredoxin. The presence of cysteine methyl ester had no effect on waving with intact thioredoxin.

Waving obtained with reduced thioredoxin in the absence of cysteine methyl ester was comparable to that using oxidized thioredoxin. Since the reduced form of the enzyme is necessary to cleave Bunte salts, this shows that thioredoxin may be involved in something other that Bunte salt cleavage when added directly to the bisulfite waving solution. The other possibility is that thioredoxin is reduced by the bisulfite and then cleaves Bunte salts.

Example 8 - Influence of T_{31-36} and Cysteine

The effect of a secondary reductant with T_{31-36} (trp-Cys-Gly-Pro-Cys-Lys) was studied to determine whether the amount of waving obtained with the minimal peptide could be increased. T_{31-36} is one of the minimal active site peptides and is not limited by molecular size as is intact thioredoxin. Cysteine was used as the secondary reductant in the presence of reduced T_{31-36} (2 uM) and 3.5% bisulfite. Cysteine itself gave increased waving in the uM range. However, the presence of both T_{31-36} and cysteine (10 uM) in bisulfite showed no increase in waving as compared to bisulfite alone.

Example 9 – Hair Straightening

The following formula, or obvious variations thereof, incorporating thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide, can be used with known procedures to straighten hair:

	per cent
Emulsion base:	
Demineralized water	to 100.0
Cetyl alcohol emulsified by oxyethylated cetyl	

alcohol	22.0
Demineralized water	30.0
Sodium carbonate glycinate	5.0
Ammonium thioglycollate or thiolactate (50% aqueous soln)	3.0
EDTA (disodium salt)	0.3
Sodium p-hydroxybenzoate methyl ester	0.05
Monoethanolamine	2.0
Imidazoline	0.2
Perfume	0.2
Thioredoxin or a thioredoxin-derived, or thioredoxin-like dithiol peptide	1-100 nmole/ml

Example 10 – Hair Removal

The following formula, or obvious variations thereof, incorporating thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide, can be used with known procedures to remove hair:

	per cent
Sodium picosulfate	6.5
Calcium thioglycollate	1.5
Calcium hydroxide	7.0
Sodium laurel sulfate	0.02
Sodium silicate '0'	3.43
Thioredoxin or a thioredoxin-derived, or thioredoxin-like	

dithiol peptide	1-100 nmole/ml	
Perfume	q.s.	
Distilled water	to	100.0

Procedure: Heat the water to 70°C. With stirring add the sodium laurel sulfate and sodium picosulfate; continue stirring until melted and dispersed. Discontinue heating and cool/stir to room temperature. Add the calcium hydroxide and perfume. Add the calcium thioglycollate and thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide and stir until uniform.

Example 11 – Hair Softening

Thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide can be incorporated, advantageously, into a standard lather shaving cream or brushless shaving cream to soften the hair. This softening of the hair complements the softening realized by the soap and water contact with such shaving creams. The level of thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide used in such shaving creams can be varied as described above. Likewise, the percentage of soaps of such standard shaving creams can be reduced with the use of the compounds of the invention. The particular levels of soap, thioglycolic acid compound, and thioredoxin or a thioredoxin-derived, or thioredoxin-like dithiol peptide can be readily adjusted by a person skilled in the art to meet the requirements for softening different types of hair.

Example 12 – Animal Hair

The compositions and processes of the previous examples can be readily adapted by a person skilled in the art to be used to care for animal hair in general, e.g., dog, cat, horse, and the like.

Claims

1. A composition of matter for waving, straightening, removing, or softening human hair which comprises thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide and

(a) an organic reductant compound; or

(b) a sulfite or bisulfite compound.

2. A composition of matter, according to claim 1, which comprises an organic reductant compound and thioredoxin, or a thioredoxin-derived, or a thioredoxin-like dithiol peptide.

3. A composition of matter, according to claim 1, wherein said organic reductant compound is present at a concentration of about 0.01 M to about 1.0 M, and said thioredoxin or thioredoxin-derived, or thioredoxin-like, dithiol peptide is present at a concentration of about 1 nmole/ml to about 100 nmole/ml.

4. A composition of matter, according to claim 1, which comprises a sulfite or bisulfite compound and thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide.

5. A composition of matter, according to claim 1, wherein said sulfite or bisulfite compound is present at a concentration of about 0.1 M to about 1.0 M, and said thioredoxin or thioredoxin-derived, or thioredoxin-like, dithiol peptide is present at a concentration of about 0.1 umole/ml to about 100 umole/ml.

6. A composition of matter, according to claim 1, wherein said thioredoxin-derived dithiol peptide is the fragment containing residues 1 through 37 produced by cyanogen bromide cleavage of E. coli thioredoxin.

7. A composition of matter, according to claim 1, wherein said thioredoxin-derived dithiol peptide is the fragment containing residues 19-36 produced by trypsin digestion of residues 1 through 37 produced by cyanogen bromide cleavage of E. coli thioredoxin.

8. A composition of matter, according to claim 1, wherein said organic reductant compound is selected from the group consisting of thioglycolic acid, L-cysteine ethylester, beta-mercaptoethylamine, cysteine, mercaptosuccinic acid, beta-mercapto propionic acid, dimercapto adipic acid, thiomalic acid, thioglycollamides, glycol thioglycollate, glycerol thioglycollate, thiolactic acid and salts thereof.

9. A thioredoxin-derived, or thioredoxin-like, dithiol peptide comprising the redox-active peptide sequence Cys-X-Y-Cys, Cys-X-Y-Cys-Lys, or Trp-Cys-X-Y-Cys-Lys, wherein X and Y, independently, can be any of the natural 20 amino acids.

10. A thioredoxin-derived, or thioredoxin-like, dithiol peptide, according to claim 9, comprising the redox-active peptide sequence Cys-Gly-Pro-Cys, Cys-Gly-Pro-Cys-Lys, or Trp-Cys-Gly-Pro-Cys-Lys.

11. A process for waving, straightening, removing, or softening human hair which comprises applying to said human hair a composition comprising thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide and

(a) an organic reductant compound; or

(b) a sulfite or bisulfite compound.

12. A process, according to claim 11, wherein said composition comprises an organic reductant compound and thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide.

13. A process, according to claim 11, wherein said organic reductant compound is present at a concentration of about 0.01 M to about 1.0 M, and said thioredoxin or thioredoxin-derived, or thioredoxin-like dithiol peptide is present at a concentration of about 1 nmole/ml to about 100 nmole/ml.

14. A process, according to claim 11, which comprises applying to said human hair a composition comprising a sulfite or bisulfite compound and thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide.

15. A process, according to claim 11, wherein said sulfite or bisulfite compound is present at a concentration of about 0.1 M to about 1.0 M, and said thioredoxin or thioredoxin-derived, or thioredoxin-like dithiol peptide is present at a concentration of about 1 nmole/ml to about 100 nmole/ml.

16. A process, according to claim 11, wherein said thioredoxin-derived, or thioredoxin-like dithiol peptide is the fragment containing residues 1 through 37 produced by cyanogen bromide cleavage of E. coli thioredoxin.

17. A process, according to claim 11, wherein said thioredoxin-derived, or thioredoxin-like dithiol peptide is the fragment containing residues 19 through 36 produced by trypsin digestion of residues 1 through 37 produced by cyanogen bromide cleavage of E. coli thioredoxin.

18. A process, according to claim 11, wherein said thioredoxin-derived, or thioredoxin-like, dithiol peptide comprises the redox-active peptide sequence Cys-X-Y-Cys, Cys-X-Y-Cys-Lys, or Trp-Cys-X-Y-Cys-Lys, wherein X and Y, independently, can be any of the natural 20 amino acids.

19. A process, according to claim 18, wherein said redox-active peptide sequence is Cys-Gly-Pro-Cys, or Cys-Gly-Pro-Cys-Lys, or Trp-Cys-Gly-Pro-Cys-Lys.

20. A process, according to claim 11, wherein said organic reductant compound is selected from the group consisting of thioglycolic acid, L-cysteine ethylester, beta-mercaptoethylamine, cysteine, mercaptosuccinic acid, beta-mercapto propionic acid, dimercapto adipic acid, thiomalic acid, thioglycollamides, glycol thioglycollate, glycerol thioglycollate, thiolactic acid and salts thereof.

21. A composition of matter for waving, straightening, removing, or softening animal hair which comprises thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide and

(a) an organic reductant compound; or

(b) a sulfite or bisulfite compound.

22. A composition of matter, according to claim 21, wherein said organic reductant compound is selected from the group consisting of thioglycolic acid, L-cysteine ethylester, beta-mercaptoethylamine, cysteine, mercaptosuccinic acid, beta-mercapto propionic acid, dimercapto adipic acid, thiomalic acid, thioglycollamides, glycol thioglycollate, glycerol thioglycollate, thiolactic acid and salts thereof.

23. A process for waving, straightening, removing, or softening animal hair which comprises applying to said animal hair a composition comprising thioredoxin or a thioredoxin-derived, or thioredoxin-like, dithiol peptide and

(a) an organic reductant compound; or

(b) a sulfite or bisulfite compound.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/04694

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 7/09

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0183506 (REPLIGEN CORP.) 4 June 1986, see the whole document	1-23

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[10] Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th April 1989	- 3 MAY 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	L. ROSSI

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8804694 SA 26280

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 27/04/89 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0183506	04-06-86	JP-A- 61137899	25-06-86
		US-A- 4738841	19-04-88